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Extreme Drug Resistance Assay and Response to Chemotherapy in Patients with Primary Peritoneal Carcinoma

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Background and Objectives: The extreme drug resistance (EDR) assay is an in vitro chemoresistance assay performed on tumor samples grown in culture and is claimed to predict drugs unlikely to produce response. Its clinical value in patients with epithelial ovarian cancer (EOC) has recently been questioned. The aim of this study is to describe EDR assay results and responses to chemotherapy among women with primary peritoneal adenocarcinoma (PPA) and to compare them with those of women with EOC.

Methods: Fresh tumor specimens from 20 consecutive women with PPA were tested for EDR to the following drugs: cisplatin, carboplatin, paclitaxel, doxorubicin, cyclophosphamide, ifosfamide, etoposide, hexamethylmelamine, and topotecan. They were then treated with cisplatin-based combination chemotherapy. The results of the EDR assay and response to chemotherapy were compared with those among women with EOC.

Results: There was no significant difference in the incidence of EDR to cisplatin, carboplatin, paclitaxel, doxorubicin, cyclophosphamide, ifosfamide, etoposide, hexamethylmelamine, and topotecan among patients with PPA and those with EOC. The response rate of PPA patients to chemotherapy was 80.0% and unrelated to EDR to the individual drugs used in combination chemotherapy.

Conclusions: The EDR profile and response to cisplatin-based chemotherapy among women with PPA were similar to those among women with EOC. These findings support treating both conditions similarly. EDR to individual drugs does not preclude response to combination chemotherapy.

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KEY WORDS: **primary peritoneal carcinoma; epithelial ovarian cancer; extreme drug resistance assay; response to chemotherapy**

INTRODUCTION

Primary peritoneal adenocarcinoma (PPA), a disease that occurs exclusively in women, is an adenocarcinoma that develops from the peritoneal lining of the abdomen and pelvis, characterized by widely spread intraperitoneal cancer, uninvolved or minimally involved ovaries, and no identifiable primary. PPA has been reported following bilateral oophorectomy performed for benign disease or prophylactically and accounts for approximately 10% of cases with a presumed diagnosis of epithelial ovarian cancer (EOC) [1].

PPA has a similar clinical presentation to EOC. Stage for stage, it also has a similar prognosis to EOC [2]. Several authors have suggested that patients with PPA respond to chemotherapy similarly to patients with EOC and have recommended treating women with this type of cancer using the same chemotherapeutic regimens em-

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ployed in women with EOC [3–5]. However, these findings were not confirmed by others [6].

Interest in in vitro chemosensitivity/resistance assays has been renewed following the development of the extreme drug resistance (EDR) assay by Kern and Weisenthal in 1990 [7]. In this assay, human tumor colonies are cultured in soft agar and tested at high concentrations for long exposure times so that the coefficients for concentration × time exceed those achieved in vivo by 100-fold. Tumors not inhibited by such treatment are said to have EDR. A clinical trial has demonstrated that the EDR assay has >99% accuracy in identifying which chemotherapeutic agents will not effect a clinical response [7]. However, the clinical utility of this assay in the management of women with primary EOC has been questioned [8].

The relationship between in vitro drug sensitivity and in vivo response to chemotherapy among women with PPA is unknown. In addition, results of the EDR assay among women with PPA compared with those among women with EOC have not been previously reported. The objective of the current study is to describe the EDR assay results and response to chemotherapy among women with PPA and compare them with our previous report [8] on women with EOC.

MATERIALS AND METHODS

Twenty consecutive women with PPA, seen at Roswell Park Cancer Institute (n = 15) and the University of Vermont (n = 5) between March 1995 and September 1998, were the subject of the current study. Tumor specimens for the EDR assay were obtained from all patients during initial cytoreductive surgery before starting chemotherapy.

Following frozen section confirmation of the histological diagnosis of PPA, at least 1 g of viable tumor tissue was immersed in incubation medium and sent promptly to the reference laboratory (Onchotech, Irvine, CA), where the EDR assay was performed according to the method described by Kern and Weisenthal [7]. The test measures inhibition of DNA synthesis by calculating the rate of proliferation of tumor cells plated in soft agar medium using a thymidine incorporation method.

One specimen was obtained from each patient from a site judged to be most representative of viable tumor tissue. In all patients, tumor specimens were tested for response to the following drugs: cisplatin, carboplatin, paclitaxel, cyclophosphamide, ifosfamide, doxorubicin, etoposide, hexamethylmelamine, and topotecan. Since cyclophosphamide requires in vivo hepatic drug activation, the test utilizes its active metabolite, 4-hydroxyperoxycyclophosphamide. The assessability rate of the assay was defined as the number of tumor samples successfully grown in vitro divided by the number of tumor samples implanted.

Pathological diagnosis of PPA was made by staff at the divisions of pathology at Roswell Park Cancer Institute and the University of Vermont. The diagnosis of PPA was based on the Gynecologic Oncology Group criteria, described in the study by Bloss et al. [3]. These diagnostic criteria include: (1) ovaries either absent, physiologically normal in size with a maximum diameter <4.0 cm, or enlarged by a benign process; 2) involvement in extraovarian sites greater than that on the surface of either ovary; 3) microscopically, ovaries either not involved with tumor or exhibiting only serosal or cortical implants smaller than 5 × 5 mm; 4) histological and cytological characteristics of tumor predominantly of the serous type; and 5) no evidence of other primary cancers that might explain the peritoneal involvement.

Patients with PPA who were candidates for and accepted chemotherapy were treated with paclitaxel and cisplatin combination chemotherapy following recovery from cytoreductive surgery. This regimen consists of paclitaxel (135 mg/m^2 over 24 hr) followed by cisplatin (75 mg/m^2) every 4 weeks for 6 cycles. Patients who were unable to tolerate paclitaxel were treated with cyclophosphamide (750 mg/m^2) and cisplatin (75 mg/m^2).

All patients were surgically staged according to the FIGO criteria [9]. Chemotherapeutic response was assessed according to the World Health Organization criteria [10] and CA-125 values [11]. Performance status was assessed according to the Gynecologic Oncology Group criteria. Optimal cytoreduction was defined as surgery resulting in largest residual tumor <1 cm in diameter. Suboptimal cytoreductive surgery was defined as surgery resulting in largest residual tumor mass >1 cm in diameter. Patients were considered evaluable for response if they received at least 2 courses or demonstrated progression following 1 course of chemotherapy.

Following 6 courses of chemotherapy, patients who had complete clinical response were counseled regarding reassessment surgery. In patients who underwent reassessment surgery, complete surgical response was defined as absence of pathologically demonstrable disease among those with residual disease (gross or microscopic) after primary surgery. Partial surgical response was defined as a decrease, by ≥50% of residual disease following primary surgery. Surgically stable disease was defined as no change in the size of residual disease or <50% decrease in size of residual disease following primary surgery. Progressive disease was defined as new areas of growth or an increase of known residual tumor noted at reassessment surgery or before. Overall response was defined as complete or partial clinical and complete or partial surgical response.

The results of the EDR assay and response to chemotherapy among women with PPA were compared with the results of those among women with primary EOC, which have been previously published [8]. The method

TABLE I. Characteristics of Patients with Primary Peritoneal Cancer Enrolled in the Study

Median (range) age in years	65 (36–84)
Histology	
Papillary serous	18 (90%)
Mucinous	2 (10%)
Stage	
III	16 (80%)
IV	4 (20%)
Grade	
1	1 (5%)
2	6 (30%)
3	13 (65%)
Cytoreduction	
Optimal	14 (70%)
Suboptimal	6 (30%)
Median (range) performance status	0 (0–2)

TABLE II. Extreme Drug Resistance Profile of Women with Primary Peritoneal Cancer and Those with Epithelial Ovarian Cancer

Test result	Ovarian cancer (n = 94)[a]	Primary peritoneal cancer (n = 19)[a]
Extreme drug resistance to		
Cisplatin	4 (4.3%)	1 (5.3%)
Carboplatin	8 (8.5%)	4 (21.1%)
Paclitaxel	21 (22.3%)	5 (26.3%)
Doxorubicin	44 (46.8%)	7 (36.8%)
Cyclophosphamide	15 (16.0%)	2 (10.5%)
Ifosfamide	2 (2.1%)	0
Etoposide	14 (14.9%)	2 (10.5%)
Hexamethylmelamine	10 (10.6%)	1 (5.3%)
Topotecan	10 (10.6%)	1 (5.3%)
None of the above	14 (14.9%)	3 (15.8%)
More than 1 of the above	37 (39.4%)	7 (36.8%)

[a]These numbers reflect the number of tumor samples successfully grown in culture. Using χ^2 analysis, there was no significant difference between the 2 groups.

for collection of samples for the EDR assay, the drugs tested, the methodology of the assay, the chemotherapy regimens used, and the methods of assessment of response to chemotherapy employed in the current study were identical to those of the previous study [8] used for comparison. Statistical analysis was performed using the Fisher exact test and the χ^2 test. Two-tailed P values <0.05 were considered statistically significant.

RESULTS

Twenty consecutive women with PPA were enrolled in the study. The EDR assay could not be performed on 1 primary peritoneal tumor specimen because of low growth rate. Thus, the assessability rate for the primary peritoneal tumors was 95% (19/20). The assessability rate for the primary peritoneal cancer was similar to the previously reported 94% assessability rate for EOC [8].

Table I describes the characteristics of PPA patients enrolled in the study. Table II describes the results of the EDR assay among women with PPA and compares them with those previously described [8] for women with EOC. Using χ^2 analysis, there was no statistically significant difference in the results of the EDR assay between women with PPA and those with EOC. As demonstrated in Table II, doxorubicin was the drug that most commonly demonstrated EDR in both groups (46.8% for EOC and 36.8% for PPA). Ifosfamide and cisplatin were the drugs that least commonly demonstrated EDR among patients with EOC and those with PPA (2.1% vs. 0% and 4.3% vs. 5.3%, respectively). One tumor sample demonstrated EDR to cisplatin, 5 demonstrated EDR to paclitaxel, and 2 demonstrated EDR to cyclophosphamide.

As demonstrated in Table II, the incidence of EDR to carboplatin was >2-fold higher among patients with PPA compared to patients with EOC (21.1% vs. 8.5%, respectively). In both groups, the median number (range) of drugs to which tumors demonstrated EDR was 1 (0–5).

Twenty patients with PPA received postoperative cisplatin-based combination chemotherapy: 18 (90%) received paclitaxel and cisplatin and 2 (10%) received cyclophosphamide and cisplatin. The median number of chemotherapy courses was 6 and the range, 2–9. All patients with PPA were evaluable for response to chemotherapy. Surgical assessment of response was performed in 12 (60.0%) patients: 8 by laparotomy and 4 by laparoscopy. Response to chemotherapy was assessed clinically in 8 patients (40.0%). As demonstrated in Table III, the overall response rate of women with PPA to cisplatin-based chemotherapy was 80.0% (16/20), similar to our previously reported 85.3% (64/75) overall response rate among women with EOC using similar chemotherapeutic regimens ($P = 0.45$) [8].

In the current study, the single woman whose tumor demonstrated EDR to cisplatin had a partial clinical response to paclitaxel and cisplatin. Tumor samples obtained from 5 women with PPA demonstrated EDR to paclitaxel, and 2 of these 5 tumors also demonstrated EDR to cyclophosphamide. These 5 women had the following response to paclitaxel and cisplatin chemotherapy: 1 each with complete surgical response, partial clinical response, and progression and 2 with complete clinical response.

DISCUSSION

To our knowledge, the current study is the first to report on EDR assay results among women with PPA and compare them with those of women with EOC. In the current study we used our previous report [8] on EDR among women with EOC as control. The methodologies applied in the 2 studies were identical. Some of the patients included in the current study were previously included in studies from Roswell Park Cancer Institute

TABLE III. Response of Women with Primary Peritoneal Carcinoma to Cisplatin-Based Chemotherapy

Response	Number (%)
Surgical	
Complete response	4 (20.0%)
Partial response	6 (30.0%)
Stable disease	2 (10.0%)
Progression	0
Clinical	
Complete response	4 (20.0%)
Partial response	2 (10.0%)
Stable disease	0
Progression	2 (10.0%)
Overall response[a]	16 (80.0%)

[a]Overall response was calculated by adding complete and partial surgical responses and complete and partial clinical responses.

reporting on the response of women with PPA [12] to chemotherapy.

Several investigators have demonstrated that women with PPA have chemotherapeutic response rates similar to those with EOC [3–5]. Lele et al. [4] reported an overall response rate of 65% in a group of 23 women with PPA treated primarily with cisplatin-based regimens following cytoreductive surgery. This response rate was comparable to that achieved by the same authors with similar combinations in patients with ovarian cancer [4]. In a case-control study of 33 patients with PPA and 33 patients with papillary serous ovarian cancer, Bloss et al. [3] found no significant difference between cases and controls with regard to response to chemotherapy. In contrast, in a case-control study, Killackey and Davis [6] found PPA patients to have a poorer response to cisplatin-based regimens than those with EOC.

Testing EDR in patients with PPA and correlating the assay results to response to chemotherapy might be of importance because of the polyclonal nature of some PPAs. Some might argue that this assay may not be useful in these patients and more appropriate for predicting response in monoclonal ovarian cancer. However, our study did not find a difference between EDR assay results or response to chemotherapy between patients with PPA and those with EOC.

The EDR assay has been developed as an exclusion test to identify drugs unlikely to demonstrate response [7]. The test meets certain basic criteria for assay utility. The assessability rates for ovarian and primary peritoneal cancers (94% and 95%, respectively) are high, the test results are usually available within a relatively short period of time (1 week), and the test information is easily interpreted. However, the value of the EDR assay in the clinical management of patients with EOC has been questioned [8,13]. In our previous study correlating EDR to response to paclitaxel and cisplatin given as first-line chemotherapy in patients with EOC, we found that EDR to paclitaxel did not preclude response to the combination of paclitaxel and cisplatin [8]. Similarly, we found, in the current study, that 1 and 4 of 5 PPA patients whose tumor demonstrated EDR to cisplatin and paclitaxel, respectively, demonstrated response to cisplatin and paclitaxel combination chemotherapy.

The current study demonstrates that women with PPA have an EDR profile similar to that of women with EOC. Since clinical correlation between EDR and response to chemotherapy has been demonstrated by some authors [7], the results of the current study might by supportive of the current practice of treating women with PPA in a manner similar to those with EOC. However, the limitation imposed by the small study population should be considered. It is possible that with a larger study population some significant differences between the EDR profiles between women with PPA and those with EOC might have been found. It might be argued that the difference in EDR to carboplatin between women with EOC and those with PPA demonstrated in the current study (8.5% vs. 21.1%, respectively) might have reached statistical significance with a larger study population. However, that difference was demonstrated in only 1 drug, and the remainder of the drugs tested exhibited similar EDR between both groups.

In the current study, 1 tumor demonstrated EDR to cisplatin, 5 tumors demonstrated EDR to paclitaxel, and no tumor demonstrated EDR to both paclitaxel and cisplatin. The small numbers did not permit adequate assessment of the correlation between the EDR assay results and response to chemotherapy. However, 1 patient with EDR to cisplatin and 4 of 5 patients with EDR to paclitaxel demonstrated response to the combination paclitaxel and cisplatin. This finding is in agreement with our previous finding [8] correlating EDR to paclitaxel and response to the combination paclitaxel and cisplatin in women with EOC. This finding might be supportive of testing EDR to drug combinations rather than individual drugs, as was the practice of the reference laboratory during the period of the current study.

In conclusion, the EDR profile and response to platinum-based chemotherapy among women with PPA are similar to those among women with primary EOC. These findings support the practice of treating women with PPA in a manner similar to those with EOC. EDR to an individual drug does not preclude response to combination chemotherapy utilizing the same drug.

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